Manual

Thymosin β_4 EIA

For the determination of thymosin β_4 in serum and thymus extract

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Table of Contents

1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of thymosin β_4 in serum and thymus extract.

For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

The beta-thymosins are a family of highly conserved polar 5 kDa peptides originally thought to be thymus hormones. Further studies demonstrated that beta-thymosins are ubiquitous; they have been found in a variety of tissues and cell lines. The highest concentrations have been detected in spleen, thymus, lung, and peritoneal macrophages.

Thymosin β_4 binds monomeric actin in a 1:1 complex acting as an actin buffer, preventing polymerisation into actin filaments but supplying a pool of actin monomers when needed by the cell. Changes in the expression of thymosin β_4 appear to be related to the differentiation of cells. Thymosin β_4 is detected outside of cells in blood plasma and wound fluid. Several biological effects are attributed to thymosin β_4, like induction of metallo-proteinases, chemotaxis, angiogenesis and inhibition of inflammation as well as inhibition of bone marrow stem cell proliferation.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR9520	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	1 x 100 ml
KR9520	CONJ	Conjugate, peroxidase-labelled, ready-to-use	1 x 22 ml
KR9520	AB	Antibody (Anti-Thymosin β_4 antibody)	1 x 16 ml
KR9520	STD	Standards, lyophilised (see specification for concentrations)	3 x 5 vials
KR9520	CTRL1	Control, lyophilised (see specification for range)	3 x 1 vial
KR9520	CTRL2	Control, lyophilised (see specification for range)	3 x 1 vial
KR9520	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 100 ml

Cat. No.	Label	Kit components	Quantity
KR9520	SUB	Substrate (tetramethylbenzidine), ready-to-use	2 x 15 ml
KR9520	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 *g*
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 3 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.

- The **lyophilised standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. **Reconstitution details** as well as **concentrations and ranges** are given in the **specification data sheet**.
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Serum

Serum can be used without dilution. Store samples at -20 °C.

Thymus extract

Thymus extracts have varying compositions. Please contact the supplier when using thymus extracts.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of thymosin ß$_4$ (Tß$_4$) in serum and thymus preparations.

The test principle is based on a competition between antigen in the sample or standards and the antigen coated on the wells of microplate. A peroxidase-conjugated antibody is used for detection and quantification, and tetramethylbenzidine (TMB) as a peroxidase substrate. The enzymatic reaction is terminated by acidic stop solution. The intensity of the colour is inversely proportional to the concentration of Tß$_4$. A dose response curve of absorbance unit (optical density, OD at 450 nm) vs. concentration is generated using the values obtained from standard. Tß$_4$, present in the samples, is determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/sample/controls on a protocol sheet.

Take as many microtiter strips as needed from kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	**Before use**, wash the wells **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each **50 µl standards/controls/samples** into the respective wells.
3.	Add **150 µl of AB** (antibody) into each well.
4.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) in the dark on a horizontal shaker.
5.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
6.	Add **200 µl conjugate** (CONJ) into each well.
7.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) in the dark on a horizontal shaker.
8.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
9.	Add **200 µl substrate** (SUB) into each well.
10.	Incubate for **10–20 minutes*** at room temperature (15–30 °C) in the dark.
11.	Add **50 µl stop solution** (STOP) into each well and mix well.
12.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm as a reference.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm.

1. **4 parameter algorithm**

   It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e. g. 0.001).

2. **Point-to-point calculation**

   We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. **Spline algorithm**

   We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

9. LIMITATIONS

Samples with an OD lower than the OD of the highest standard can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range

The values in healthy people are strongly age-dependent. Newborn show the highest, and from the age of 20 the serum values are falling. We recommend each laboratory to establish its own reference range for different age-ranges.

11. PRECAUTIONS

- All reagents in the kit package are for *research* use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

12. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the

test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.

- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

Used symbols:

 Temperature limitation

REF Catalogue Number

RUO For research use only

→REF To be used with

 Manufacturer

 Contains sufficient for <n> tests

LOT Lot number

 Use by

 Attention

 Consult instructions for use

 Consult specification data sheet